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L. L. Holley

M K. Heidman

Randolph Chambers
William and Mary, rmcham@wm.edu

S. Laurie Sanderson
William and Mary, slsand@wm.edu

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Mucous contribution to gut nutrient content in American gizzard shad *Dorosoma cepedianum*

L. L. HOLLEY*, M. K. HEIDMAN†, R. M. CHAMBERS† AND S. L. SANDERSON†‡

**Edge Engineering and Science, 16360 Park Ten Place, Suite 300, Houston, TX 77084, U.S.A.,*

†Department of Biology, College of William and Mary, P .O. Box 8795, Williamsburg, VA 23187, U.S.A.

‡Author to whom correspondence should be addressed. Tel.: +1 757 221 2123; email: slsand@wm.edu

Running headline: MUCUS IN GUT CONTENT OF *D. CEPEDIANUM*

This study developed and applied an approach to calculate the proportion of fish gut content composed of mucus secreted by the oropharyngeal cavity and gut. The amount of nitrogen in the contents of the foregut (esophagus and gizzard) and the epibranchial organs of suspension-feeding American gizzard shad *Dorosoma cepedianum* was significantly higher than the nitrogen in the homogeneous food source. Using data collected from suspension-feeding experiments and the nitrogen content of *D. cepedianum* mucus, a series of equations illustrated that mucus constituted approximately 10% of *D. cepedianum* foregut content and 12% of epibranchial organ content by dry mass. Future quantification of fish feeding selectivity and absorption efficiency can use this approach to take into account the contribution of fish mucus to the nutrients in the gut contents. This study supports the conclusion that suspension-feeding *D. cepedianum* in a heterogeneous environment selectively ingest nutrient-rich particles, even when gut nutrient content is adjusted to take into account the contribution of mucus.

Key words: bioenergetics; epibranchial organs; feeding selectivity; filter feeding; suspension feeding.

INTRODUCTION

Several functions of fish mucus have been described, including ionic and osmotic regulation, nest building and protection, respiration, reproduction, disease resistance, excretion, communication, gas exchange, locomotion, and feeding (Shephard, 1994). Although mucus is ubiquitous in fish feeding, few studies have quantified the extent to which mucus is involved. Mucus is present in the fish alimentary tract (Wilson & Castro, 2011), but contributions of mucus to the nutrient or energy content of feces are traditionally considered to be minor and have not been factored into bioenergetics calculations of absorption efficiency (Jobling, 1994).

This assumption that fish-secreted substances have a negligible influence on the nutrient content of food in the gut is also universal in studies of feeding selectivity. For example, a high nutrient content in the gut of suspension-feeding fish has been attributed to selective sorting and swallowing of small nutrient-rich food particles (e.g., Heidman *et al.*, 2012). However, mucus secreted in the oropharyngeal cavity and other regions of the alimentary tract contains nutrients. Therefore, high nutrient levels in suspension-feeding fish foreguts may be due in part to ingestion of the fish's own mucus rather than being due solely to the selective ingestion of food particles with high nutrient content.

Ideally, analyses of particle selectivity in suspension-feeding fishes should account for the nutrients from mucus that has been ingested with food particles or secreted into the foregut. One estimate from unpublished data suggested that mucus and enzyme secretions associated with the foregut lining contributed <5% of the organic content in the gut of juvenile white sucker *Catostomus commersoni* (Lacepède 1803) (Ahlgren, 1996). No estimates for the nutrient and energy content of fish mucus in the alimentary tract have been published, even though mucus can

represent a substantial portion of an aquatic animal's energy budget (e.g., Davies & Hawkins, 1998). Fish mucus has been reported to consist of up to 10-11% nitrogen by dry weight in some species (Arnal *et al*., 2001; Arnal & Morand, 2001), suggesting that mucus has the potential to be a significant source of the nitrogen found in the gut contents of suspension-feeding fishes.

Mucus present on surfaces in the oropharyngeal cavity serves important functions in suspension-feeding fishes. For example, during hydrosol filtration in Nile tilapia *Oreochromis niloticus* (L. 1758), mucus on the gill arches and rakers captured and aggregated food particles small enough to escape through the gaps between filtering structures (Northcott & Beveridge, 1988; Sanderson *et al*., 1996). Hoogenboezem & van den Boogaart (1993) identified mucus as an important component in the accumulation, storage, and transport of food particles in suspension-feeding freshwater bream *Abramis brama* (L. 1758). Large numbers of zooplankton were contained in mucus boluses in the dissected oropharyngeal cavities of *A. brama*. In addition, Paig-Tran & Summers (2014) used histology to detect mucus-producing cells on the filter of three suspension-feeding species in the ray family Mobulidae, suggesting that the mucus assists in filtration or particle transport.

The facultative suspension-feeding American gizzard shad *Dorosoma cepedianum* (Lesueur 1818) consumes zooplankton and phytoplankton when these live foods are available, and consumes benthic detritus when plankton are scarce (Mundahl & Wissing, 1988). Goblet cells and mucus are common throughout the *D. cepedianum* alimentary tract, including the oropharyngeal cavity, epibranchial organs, esophagus, and gizzard (Heinrichs, 1982). In *D. cepedianum*, epibranchial organs are paired sacs in the posterior oropharynx (Fig. 1) thought to collect and consolidate food particles which are then delivered to the esophagus in a mucus

77 bolus for swallowing. Epibranchial organs have been associated with microphagy in many
78 groups of fishes, including Osteoglossiformes, Cypriniformes, Gonorhynchiformes and
79 Clupeiformes (Schmitz & Baker, 1969; Kapoor *et al*., 1975). The epibranchial organ contents
80 of microphagous fishes, however, have not been quantified in previous studies. Drenner *et al*.
81 (1982) found plankton bound with mucus in *D. cepedianum* epibranchial organs, but *D.*
82 *cepedianum* were not observed to use mucus to trap particles in the oropharyngeal cavity
83 during crossflow filtration (Sanderson *et al*., 2001). *Dorosoma cepedianum* intraoral mucus may
84 function to aggregate particles in the posterior oropharynx and epibranchial organs or regulate
85 the loss of water between the rakers and between the gill arches (Smith & Sanderson, 2007).
86 Given the substantial importance of fish mucus in suspension feeding, the purpose of this
87 study was to quantify the contribution of mucus to the gut content of fish. The objectives were to
88 (1) quantify the nutrient content (nitrogen and carbon) of mucus on external and internal
89 epithelia in *D. cepedianum*, (2) derive a series of equations to calculate the contribution of mucus
90 to gut nutrient or gut energy contents in fish, and (3) calculate the contribution of mucus to the
91 nutrients quantified in the food contents of the epibranchial organs and the foregut (esophagus and
92 gizzard) of *D. cepedianum*. This suspension-feeding species has substantial importance for
93 nutrient cycling in freshwater ecosystems (Domine *et al*., 2010; Schaus *et al*., 2010) and was
94 chosen for this research based on published reports of feeding selectivity (Higgins *et al*., 2006;
95 Smoot & Findlay, 2010a; Heidman *et al.*, 2012).
96

MATERIALS AND METHODS

SEQUENCE OF DATA COLLECTION AND ANALYSIS

Two categories of data collection are described in the sections below: (1) collection of mucus from external surfaces and internal surfaces of *D. cepedianum*, and (2) suspension-feeding experiments followed by collection of foregut contents and epibranchial organ contents, for comparison with the food suspended in the water.

Next, the data from the mucus analysis and the feeding experiments were used in the series of equations derived here to permit calculation of the contribution of mucus to the dry mass of foregut contents and epibranchial organ contents. A subset of the feeding experiment data from Heidman *et al*. (2012) was also used as supplemental input in the equations for calculating the proportion of gut contents contributed by mucus. Subsequently, the calculations of % mucus in the foregut were applied to previously published reports of feeding selectivity in *D. cepedianum*, to determine whether published data continue to demonstrate selection of high-nutrient particles by this species even when the nutrient values in gut contents are adjusted to exclude the potential contribution of mucus identified here.

FISH COLLECTION

Adult *D. cepedianum* (range 19.0-28.0 cm standard length) were collected from rivers and lakes on the Virginia coastal plain using electrofishing techniques. Fish were maintained and fed

daily in 284 l glass holding aquaria at 19-21° C and acclimated to laboratory conditions for a minimum of five days prior to experiments.

MUCUS COLLECTION

To determine the nitrogen and carbon content of *D. cepedianum* mucus, eleven fish were euthanized by severing the vertebral column directly posterior to the cranium, followed by pithing. Due to potential contamination from the nitrogen in MS-222, this compound was not used for euthanasia.

External mucus was collected immediately post-euthanasia by sliding a flexible rubber-tipped probe gently along the flanks of the fish to separate mucus from the scale surface. With another probe, internal mucus was collected separately from surfaces within the oropharyngeal and opercular cavities, including the gill arches, gill rakers, gill filaments, and internal suspensorium. Mucus was placed onto tared Flat Tin Disks (PerkinElmer, Inc.; www.perkinelmer.com). For the internal mucus collection, mucus from two or three fish was pooled on each tin disk to ensure an adequate dry mass of mucus for analysis (n = 5).

SUSPENSION-FEEDING EXPERIMENTS

Experiments quantified the amount of nitrogen and carbon in a homogeneous food source compared with the amount of nitrogen and carbon in the contents of the epibranchial organs and foregut from feeding fish. These data were then used in the equations presented below to calculate the proportion of gut contents contributed by mucus.

Big Strike fish food pellets (Southern States Cooperative; www.southernstates.com) were milled and sieved using market grade sieves with mesh no. 120 (125 μm) and no. 60 (250 μm) (Dual Manufacturing Co., Inc.; www.dualmfg.com). This process provided uniform particle sizes with uniform nutritional quality. The experimental design of Sanderson *et al*. (1998) and Sanderson & Cech (1995) was modified to maintain a homogeneous mixture of these particles suspended within the aquarium. Four model PE-A submersible water pumps (150 l h^{-1}) (Little Giant Pump Co.; www.lg-outdoor.com) were placed in the corners of a 110 l glass aquarium containing 70 l of water. Pairs of pumps were attached to opposite ends of a perforated plastic tubing. Air stones (15 cm length) were placed along the bottom of the aquarium. The pumps and air stones created currents that prevented food particles from settling and maintained a homogeneous distribution of particles. These currents did not alter the swimming movements of the fish. Two *D. cepedianum* were transferred from the holding aquaria to the experimental aquarium 24 h prior to the start of a trial, allowing fish to acclimate and to empty the foregut of all contents. In preliminary experiments, 24 h was sufficient for complete gastric emptying. Any observable feces were siphoned from the experimental aquarium prior to each trial.

Each trial (n = 5) began by adding 10.00 g Big Strike brand food particles (125-250 μm) mixed in 50 ml of water to the aquarium. Fish were allowed to feed for one hour. To quantify the food available, water samples were taken at 2, 30, and 60 min after particles had been added. An open plastic tube (2.5 cm diameter) was pushed down through the water column onto a rubber stopper placed randomly on the bottom of the aquarium, resulting in a sealed water column sample of approximately 125 ml.

At the end of each trial, a fish chosen at random was euthanized as described previously and was dissected immediately. The foregut (esophagus and gizzard, Fig. 1) was excised within 3-5

min and was placed on a clean paper towel. The entire contents of the esophagus and gizzard were extracted using blunt, flat forceps to lift the contents without scraping the foregut lining. Foregut contents were placed in a vial containing deionized water. The entire contents of both epibranchial organs, if any, were also collected and placed in a separate vial. All samples from the feeding experiments were filtered onto tared 25 mm glass Whatman GF/C microfiber filters (General Electric Co.; www.gelifesciences.com) for nitrogen and carbon analysis.

Data from four additional feeding trials using a homogeneous food source were obtained from an experiment conducted with Big Strike brand food sieved to a smaller particle size of 75-125 µm (Heidman *et al*. 2012). These data were also used in the series of equations outlined below.

ELEMENTAL ANALYSIS

Samples from the mucus collection and from the feeding experiments were stored in a drying oven at 60 °C for at least 24 h before dry mass was measured to the nearest 0.01 mg on an AD 6 microbalance (PerkinElmer, Inc.; www.perkinelmer.com). Percent nitrogen (%N) and percent carbon (%C) by dry mass of each of the samples were determined with a 2400 Analyzer (PerkinElmer, Inc.; www.perkinelmer.com) calibrated with an acetanilide standard (71.09 %C, 10.36 %N, measurement accuracy to within 5%).

In preliminary experiments, samples of mucus and Big Strike brand food particles were placed in a muffle furnace at 450° C for 3 h to burn off organic matter. Inorganic C was then measured using the elemental analyzer and subtracted from the total C yield to determine the amount of organic C in each sample. Inorganic C was not detectable in mucus and represented less than one standard deviation of the mean total C quantified in the Big Strike brand food

188 particles (0.7% inorganic C ± 0.2, mean ± S.D., n = 9). Therefore, inorganic C was considered to
189 be negligible in mucus and commercial food.
190
191 CONTRIBUTION OF MUCUS TO FOREGUT AND EPIBRANCHIAL ORGAN
192 CONTENT
193
194 Based on the premise that the nitrogen quantified in the contents of the foregut and
195 epibranchial organs during the feeding experiments originated from either internal mucus or food
196 particles, a series of equations was derived to permit calculation of the contribution of mucus to
197 the dry mass of foregut content (n = 9) and epibranchial organ content (n = 5) for each individual
198 fish in the feeding experiments. This approach required a uniform, homogenized food source so
199 that fish could not feed selectively on particles with a higher nutrient content.
200 In the equations below, the term "epibranchial organs" can be substituted for the term
201 "foregut". All percentage terms (%) are by dry mass.
202 Known Variables:
203 N_{mucus} = mean % nitrogen of internal mucus
204 N_{food} = mean % nitrogen of food particles (Calculated as the mean of the water samples in the
205 aquarium during each trial using ground Big Strike pellets.)
206 $N_{foregut}$ = % nitrogen of foregut contents
207 $W_{foregut}$ = dry mass of the foregut contents
208 Unknown Variables:
209 W_{mucus} = dry mass of internal mucus in the foregut
210 W_{food} = dry mass of food particles in the foregut

% food = proportion of foregut content attributable to food

% mucus = proportion of foregut content attributable to internal mucus

Eq. (1) defines the relationship between the dry mass of the foregut contents, food, and mucus assuming the only substances found in the foregut are food and mucus:

$$W_{\text{mucus}} = W_{\text{foregut}} - W_{\text{food}} \quad (1)$$

Eq. (2) defines the relationship between % nitrogen and dry mass of mucus, food, and foregut contents:

$$N_{\text{mucus}}W_{\text{mucus}} + N_{\text{food}}W_{\text{food}} = N_{\text{foregut}}W_{\text{foregut}} \quad (2)$$

Substituting Eq. (1) into Eq. (2):

$$N_{\text{mucus}}(W_{\text{foregut}} - W_{\text{food}}) + N_{\text{food}}W_{\text{food}} = N_{\text{foregut}}W_{\text{foregut}} \quad (3)$$

Expanding and simplifying Eq. (3):

$$N_{\text{mucus}}W_{\text{foregut}} - N_{\text{mucus}}W_{\text{food}} + N_{\text{food}}W_{\text{food}} = N_{\text{foregut}}W_{\text{foregut}} \quad (4)$$

$$W_{\text{food}}(N_{\text{food}} - N_{\text{mucus}}) = W_{\text{foregut}}(N_{\text{foregut}} - N_{\text{mucus}}) \quad (5)$$

By setting W_{foregut} to 100% and solving for W_{food} in Eq. (5), W_{food} is converted into a proportion of food (% food) found in the foregut contents:

$$\%\ \text{food} = (N_{\text{foregut}} - N_{\text{mucus}})(N_{\text{food}} - N_{\text{mucus}})^{-1} \quad (6)$$

Eq. (7) can be used to find % mucus constituting the foregut contents using the value for % food from Eq. (6). The figure of 100% represents the entire foregut contents based on the assumption that the only substances found in the foregut during the feeding experiments are food and mucus.

$$\%\ \text{mucus} = 100\% - \%\ \text{food} \quad (7)$$

The approach presented above does not take into account absorption of nutrients that might occur in the epibranchial organs or foregut during the feeding experiments, as data are not

available on such processes. Similarly, enzymes secreted in the fish alimentary tract have not been included in this study but may contribute to the nutrients quantified in the gut contents. Pepsin, lipase, amylase, and rennin have been documented qualitatively in the gizzard of gizzard shad (Bodola, 1966), but the small amounts of material present in the esophagus and gizzard have precluded quantitative assays for digestive enzyme activity (Smoot & Findlay, 2000).

DATA ANALYSIS

Levene's tests for homogeneity of variance and Shapiro-Wilk tests for normality were performed. Values for %N and %C were not arcsine transformed, as arcsine transformation is not recommended for percentage data that do not arise from count data (Sahu, 2013). Next, the %N and %C in the water samples collected from the aquaria at 2, 30, and 60 min during the feeding experiments were analyzed using repeated measures ANOVA. These nutrient levels of the food available to the fish in the feeding experiments were then compared to the nutrient levels in the foregut and epibranchial organs using paired *t*-tests. As the internal and external mucus samples were not paired, the %N and %C content of the internal and external mucus samples were compared using one-way ANOVA. All statistical tests were performed using JMP 10 Mac (SAS Institute, Inc.; www.sas.com) at a level of significance of $P < 0.05$. A sequential Bonferroni correction was used to account for the number of statistical tests performed (Rice, 1989).

QUANTITATIVE ASSESSMENT OF PARTICLE SELECTIVITY IN FISHES

Using the above equations to calculate the contribution of mucus to the nutrients in the foreguts of suspension-feeding and detritivorous fishes, the ability of these species to selectively ingest food particles with higher nutrient value can be assessed quantitatively. For example, nutrients from *D. cepedianum* mucus can now be taken into account and subtracted from Heidman *et al*.'s (2012) calculations of *D. cepedianum* feeding selectivity. For this purpose, a conservative scenario was used in the current study where the upper limits of the 95% confidence interval for the mean percent mucus by dry mass in the foregut content and the epibranchial organs, obtained using the above methods, were used to calculate and subtract the dry mass of nutrients contributed by mucus in the foregut and epibranchial organs. In this manner, the values reported by Heidman *et al*. (2012) for *D. cepedianum* feeding selectively in the laboratory on a heterogeneous distribution of low-nutrient particles (sediment) and high-nutrient particles (ground commercial fish food) were adjusted to exclude the potential contribution of mucus in calculations of feeding selectivity. One-way ANOVAs were then performed to test for significant differences between the values of %N or the values of %C in the heterogeneous food source (particles suspended in the aquarium water or particles allowed to settle on the aquarium bottom) vs. values for the foregut and epibranchial organs from Heidman *et al*. (2012) that were adjusted using the procedure described here.

RESULTS

SUSPENSION-FEEDING EXPERIMENTS

278 Each trial in the feeding experiments using a homogeneous food source provided a value of
279 $W_{foregut}$ and values of N_{food} and $N_{foregut}$ for the above equations. The nitrogen and carbon
280 composition of the water column samples collected 2, 30, and 60 min after the addition of food
281 particles to the aquarium were not significantly different (repeated measures ANOVAs, %N $F_{2,8}$
282 = 0.64, %C $F_{2,8}$ = 0.81, $P > 0.05$). Therefore, the three nitrogen values from the water column
283 were averaged within each trial and the mean was used as the percent nitrogen of the food source
284 in the above equations (N_{food}). This stability of the nitrogen and carbon levels in the water
285 indicates that the pumps and air stones maintained a homogeneous suspension of particles in the
286 aquarium and that the food source available to the fish was effectively uniform throughout each
287 trial.
288 As food particles are thought to be temporarily stored in the form of boluses in the
289 epibranchial organs and then transported into the esophagus by muscular action (Nelson, 1967;
290 Miller, 1969; Schmitz & Baker, 1969), the epibranchial organs of some individuals were empty
291 when dissected in the feeding experiments. In these cases, the fish had food particles in the
292 foregut only. For this reason, the sample size for the foregut is larger than the sample size for the
293 epibranchial organs.
294 Relative to the %N of the homogeneous food source used in the feeding experiments, the %N
295 was significantly higher in the foregut (one-tailed paired *t*-test, $t_8 = 2.07$, $P < 0.05$) and the
296 epibranchial organs ($t_4 = 8.51$, $P < 0.0005$, Table I). Attributing this difference in nitrogen
297 content between the external food source and internal samples to mucus, the above equations can
298 be used to calculate the contribution of mucus to the contents of the foregut and epibranchial
299 organs. In contrast, the %C in the foregut and epibranchial organs was not significantly different

from that of the food source (one-tailed paired *t*-tests, foregut $t_7 = -1.74$, epibranchial $t_4 = 0.89$, $P > 0.05$, Table I) and therefore was not used to calculate mucus contribution.

ELEMENTAL ANALYSIS

The %C and %N per gram dry mass of internal mucus were 44.93 ± 1.05 (mean ± S.D., $n = 5$, range 44.02-46.59) and 10.48 ± 0.15 (mean ± S.D., $n = 5$, range 10.28-10.62), respectively. Similarly, the %C and %N per gram dry mass of external mucus were 46.05 ± 1.37 (mean ± S.D., $n = 10$, range 42.45-47.11) and 10.35 ± 0.26 (mean ± S.D., $n = 10$, range 10.06-10.82), respectively. The composition of internal mucus did not differ significantly from the composition of external mucus (one-way ANOVAs, %N $F_{1,13} = 0.33$, %C $F_{1,13} = 0.58$, $P > 0.05$).

CONTRIBUTION OF MUCUS TO FOREGUT AND EPIBRANCHIAL ORGAN CONTENT

The contribution of mucus to the dry mass of foregut and epibranchial organ content was calculated using data from the feeding experiments with a homogeneous food source, given the assumption that all nutrients in the foregut and epibranchial organs originated from either internal mucus or ingested food. Since the carbon content was similar in mucus, food, foregut, and epibranchial organs (above), nitrogen rather than carbon was used to calculate the contribution of mucus to *D. cepedianum* gut contents. Based on data from the mucus collection and the feeding experiments, the series of equations derived in this study was used to determine

322 that internal mucus constituted 10.08 ± 4.46% (mean ± S.E., n = 9) of the foregut content and
323 11.76 ± 1.15% (mean ± S.E., n = 5) of the epibranchial organ content by dry mass (Fig. 2).
324
325 QUANTITATIVE ASSESSMENT OF PARTICLE SELECTIVITY IN FISHES
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327 Results from the current study can be used to adjust the values reported by Heidman *et al*.
328 (2012), to exclude the potential contribution of mucus in their previous calculations for *D.*
329 *cepedianum* feeding selectively on a heterogeneous distribution of low-nutrient particles
330 (sediment) and high-nutrient particles (ground commercial fish food). Using the upper limits of
331 the 95% confidence interval determined here for the mean percent mucus by dry mass of the
332 foregut content (20.36%) and the epibranchial organ content (14.95%), a conservatively high
333 estimate was calculated for the dry mass of nutrients contributed by mucus. This estimate of
334 mucus contributions was then subtracted from the foregut and epibranchial organ nutrient values
335 reported previously by Heidman *et al*. (2012). Even after this adjustment to take into account the
336 nutrients contributed by mucus, there were still significant differences between the values of %N
337 or the values of %C in the heterogeneous food source (suspended in the aquarium water or
338 settled on the bottom of the aquarium) vs. the adjusted nutrient values for the contents of the
339 foregut and epibranchial organs (one-way ANOVA, Table II). The particle selectivity reported
340 by Heidman *et al*. (2012) for *D. cepedianum* suspension feeding on a mixture of low-nutrient and
341 high-nutrient particles is not attributable to nutrients in mucus ingested by the fish.
342
343 **DISCUSSION**
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347 FISH MUCUS AS A FOOD SOURCE

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349 Fish mucus contains glycoproteins and serves a nutritive function for fish in a number of
350 circumstances. For example, parent-touching behavior has been reported in a diversity of fish
351 species, often for the apparent function of consuming epidermal mucus secreted by the parent
352 (Noakes, 1979; Buckley *et al*., 2010). In addition, mouthbrooding cichlid species produce
353 intraoral mucus that is hypothesized to provide nutrition for developing young (e.g., Iq & Shu-
354 Chien, 2011). Mouthbrooding Mozambique tilapia *Oreochromis mossambicus* (Peters 1852)
355 produce a diversity of chemically distinct mucins that vary seasonally with their breeding cycle
356 (Varute & Jirge, 1971). Functional roles for these different mucins, such as antibacterial or
357 nutritive, have been proposed (Varute & Jirge, 1971).

358 Species of cleaner fish, which remove ectoparasites from "client" fish, also ingest mucus from
359 their clients' body surfaces (Gorlick, 1980; Grutter, 1997). The mucus from fifteen diverse
360 Barbadian fish species that are cleaned by gobies ranged from 6.1 to 11.6 %N by dry weight
361 (Arnal *et al*., 2001). Similarly, the %N ranged from 6.1 to 10.9 in the mucus of fifteen
362 Mediterranean fish species that are cleaned by a wrasse species (Arnal & Morand, 2001). The
363 %N of *D. cepedianum* mucus in the current study fell within the upper range of these values. The
364 weight C:N ratio quantified in mucus from four Hawaiian client fish species ranged from 3.8
365 to 4.3 (Gorlick, 1980), comparable to the C:N ratio of 4.4 for *D. cepedianum* mucus calculated in
366 the current study. These values are lower than the C:N ratio of 8-14 quantified for mucus

released by *Acropora* coral species (Wild *et al*., 2005) and 14.6 for mucus blobs released by *Aurelia* jellyfish (Dicker, 2011), indicating that fish mucus may generally be more nitrogen-rich than the mucus released by invertebrates.

FUNCTIONS OF FISH MUCUS DURING SUSPENSION FEEDING

Specifically for suspension-feeding fishes such as *D. cepedianum*, mucus can serve a number of essential functions, including food particle retention on sticky surfaces during hydrosol filtration, aggregation of particles in the posterior pharynx or epibranchial organs, and generation of inertial lift during crossflow filtration. The calculation presented here showing that mucus contributes approximately 10% of the gut nutrient content in *D. cepedianum* confirms the important roles of mucus during fish suspension feeding.

Mucus entrapment of particles is common in both vertebrate and invertebrate suspension feeders, including fish species (Sanderson & Wassersug, 1993). In suspension-feeding fishes, particles otherwise small enough to fit through the filter pores may adhere to sticky mucus (Northcott & Beveridge, 1988; Sanderson *et al*., 1996). Therefore, during hydrosol filtration (Rubenstein & Koehl, 1977; Shimeta & Jumars, 1991), mucus enables suspension-feeding fishes to trap particles that would be too small to be retained by a non-adhesive, dead-end sieve.

Distinct from hydrosol filtration, *D. cepedianum* and some other suspension-feeding fish species capture prey using crossflow filtration, during which small food particles travel in suspension parallel to the filter surface (Brainerd, 2001; Sanderson *et al*., 2001; Motta *et al*., 2010; Paig-Tran *et al*., 2013). Endoscopic video of suspension-feeding *D. cepedianum*, goldfish

389 *Carassius auratus* (L. 1758) and Singida tilapia *Oreochromis esculentus* (Graham 1928)
390 showed that particles moved independently of one another and were not trapped in mucus, as
391 they were in *O. niloticus* (Sanderson *et al*., 1996; Goodrich *et al*., 2000; Sanderson *et al*., 2001).
392 During crossflow filtration in species such as *D. cepedianum*, mucus may be present on the gill
393 arches and rakers and can still play important roles in suspension feeding even though the mucus
394 does not trap particles directly on the filter surfaces (Sanderson *et al*., 2001; Paig-Tran &
395 Summers, 2014). Such mucus can serve to aggregate food particles in the epibranchial organs or
396 the posterior pharynx directly anterior to the esophagus (Drenner *et al*., 1982; Callan & Sanderson,
397 2003). In addition, mucus present on oropharyngeal surfaces during crossflow filtration may
398 function to control water loss between filter elements (Sanderson *et al*., 2001; Smith &
399 Sanderson, 2007), thereby increasing the speed of the crossflow and the inertial lift (Belfort *et*
400 *al*., 1994; Sethi & Wiesner, 1997) that can retain particles inside the oropharyngeal cavity.
401 Only two previous studies have quantified mucus production during suspension feeding, and
402 both reported extensive variation among individuals in the amount of oropharyngeal mucus. In
403 endoscopic videotapes recorded from five suspension-feeding blue tilapia *Oreochromis aureus*
404 (Steindachner 1864), mucus was observed in the region of the gill arches for 53 ± 37% of the
405 time (Smith & Sanderson, 2007), with a range of 20% to 100% (J. C. Smith & S. L. Sanderson,
406 unpubl. data). Similarly, in three *O. niloticus* individuals, mucus presence ranged from 0.3 to
407 7.7% of the time when the fish were feeding on food that was 3-10 mm diameter and from 9.1 to
408 33.2% of the time when these individuals fed on food that was 0.1-1 mm diameter (Sanderson *et*
409 *al*., 1996). Thus, the variance in calculated contributions of mucus to gut nutrient content

410 reported here is consistent with past studies documenting substantial differences in the onset and
411 extent of mucus production among individual fish.
412
413 QUANTITATIVE ASSESSMENT OF PARTICLE SELECTIVITY IN FISHES
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415 Since multiple particles are engulfed during suspension feeding and particles are not chosen
416 individually, suspension-feeding vertebrates have been assumed to feed non-selectively
417 (Sanderson & Wassersug, 1993). However, recent studies indicate that suspension feeding and
418 detritivory in *D. cepedianum* can be a selective process (Mundahl & Wissing, 1987, 1988;
419 Higgins *et al*., 2006; Smoot & Findlay, 2010a, 2010b). Heidman *et al*. (2012) reported that *D.*
420 *cepedianum* selectively ingested particles of higher nutrient content when particles with different
421 nutrient content were distributed heterogeneously in an aquarium.
422 The conclusions of the current study raise the question: Are previously-published reports of
423 particle selectivity in suspension-feeding fishes still valid if nutrients attributed to ingested food
424 also include nutrients from mucus used to capture and retain food particles in the oropharyngeal
425 cavity? By calculating the contribution of mucus to the nutrients in the foreguts of suspension-
426 feeding and detritivorous fishes, the ability of these species to selectively ingest food particles
427 that have higher nutrient content can now be assessed quantitatively.
428 Mucus can be taken into account in the study of feeding selectivity by Higgins *et al*. (2006).
429 They reported values of nitrogen content in sediment (approximately 1.6 mg N/g dry mass
430 sample, or 0.16%) and in *D. cepedianum* foreguts (approximately 20.0 mg N/g dry mass
431 sample, or 2.0%) from Burr Oak reservoir. Using the mean value of 10.48 %N by dry mass

for *D. cepedianum* internal mucus obtained in the current study, Equations 6 and 7 presented above can be used to calculate that *D. cepedianum* feeding non-selectively on Burr Oak sediment would have foreguts containing approximately 82% sediment and 18% mucus by dry mass. Similarly, foreguts of *D. cepedianum* sampled by Higgins *et al*. (2006) at Pleasant Hill and Acton reservoirs would contain approximately 93% and 88% sediment and 7% and 12% mucus by dry mass, respectively, in the absence of selective feeding. Two of the three values calculated for percent mucus by dry mass in *D. cepedianum* foreguts from the reservoirs (18%, 12%, and 7%) under the assumption of non-selective feeding are substantially higher than the mean value (10.08%) of mucus by dry mass calculated for *D. cepedianum* foreguts in the current study, indicating that ingested mucus does not account for the nutrients in the foregut, i.e., that selective feeding had indeed occurred in the *D. cepedianum* studied by Higgins *et al*. (2006).

Finally, previously published data on *D. cepedianum* feeding selectivity can also serve as a check on the approach taken in the system of equations developed above. In addition to the above calculations using %N, the C content in *D. cepedianum* foreguts and sediment samples measured by Higgins *et al*. (2006) can be used in Equations 6 and 7 as an independent calculation for the proportion of *D. cepedianum* foreguts predicted to be composed of mucus vs. sediment in the absence of selective feeding. Using the values of organic carbon content that Higgins *et al*. (2006) quantified in sediment and *D. cepedianum* foreguts from Burr Oak (approximately 1.8% and 10.5% C, respectively), and using the mean value of 44.93 %C by dry mass of *D. cepedianum* internal mucus from the current study, Equations 6 and 7 indicate that the foregut contents of *D. cepedianum* collected at Burr Oak would be approximately 80% sediment and 20% mucus by dry mass if the fish fed non-selectively on the sediment.

454 Similarly, the foreguts of *D. cepedianum* sampled at Pleasant Hill can be estimated as 91%
455 sediment and 9% mucus by dry mass in the absence of selective feeding, and the foreguts of *D.*
456 *cepedianum* sampled at Acton can be estimated as 87% sediment and 13% mucus by dry mass.
457 As a successful test of the system of equations developed in the current study, these proportions
458 (20%, 13%, and 9%) of the *D. cepedianum* foreguts estimated to be comprised of mucus based
459 on organic C content from Higgins *et al*. (2006) are very similar to the proportions calculated
460 independently above using N content (18%, 12%, and 7%).
461 The approach presented here provides evidence that *D. cepedianum* in a heterogeneous
462 environment do selectively ingest nutrient-rich particles, even when gut nutrient content is
463 adjusted for a conservatively high estimate of mucus contributions. This approach can be applied
464 to studies of particle selectivity and absorption efficiency in other suspension-feeding fish
465 species.
466
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471 slsand and IACUC-2008-02-01-5111-slsand.
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TABLE I. Percent nitrogen and carbon by dry mass for *Dorosoma cepedianum* feeding on a homogeneous suspension of ground commercial fish food in the water column, mean ± S.D. (*n*). The % nitrogen was significantly higher in the contents of the foregut and the epibranchial organs compared to the water column, indicating a significant contribution of mucus to nitrogen levels in the foregut and epibranchial organs

Location	% Nitrogen	% Carbon
Water column	6.15 ± 0.32 (9)	44.34 ± 1.85 (8)
Foregut	6.58 ± 0.55 (9) *P* < 0.05	41.74 ± 4.31 (8) *P* > 0.05
Epibranchial organs	6.65 ± 0.29 (5) *P* < 0.0005	45.11 ± 2.30 (5) *P* > 0.05

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628

629 **TABLE II.** Results of excluding mucus from a previous report (Heidman *et al*. 2012) of
630 *Dorosoma cepedianum* feeding selectively on a heterogeneous particle distribution
631 in the laboratory. Data that exclude mucus were obtained by subtracting a
632 conservatively high estimate for the dry mass of nutrients contributed by mucus in
633 the foregut and epibranchial organs, calculated as reported in this study.
634 Continued evidence of particle selectivity in *D. cepedianum* was established by
635 significant differences between the % nitrogen and % carbon in the food (water
636 column and bottom of the aquarium) vs. the % nitrogen and % carbon in the
637 foregut and the epibranchial organs after mucus was excluded, mean ± S.D. (*n*)

638

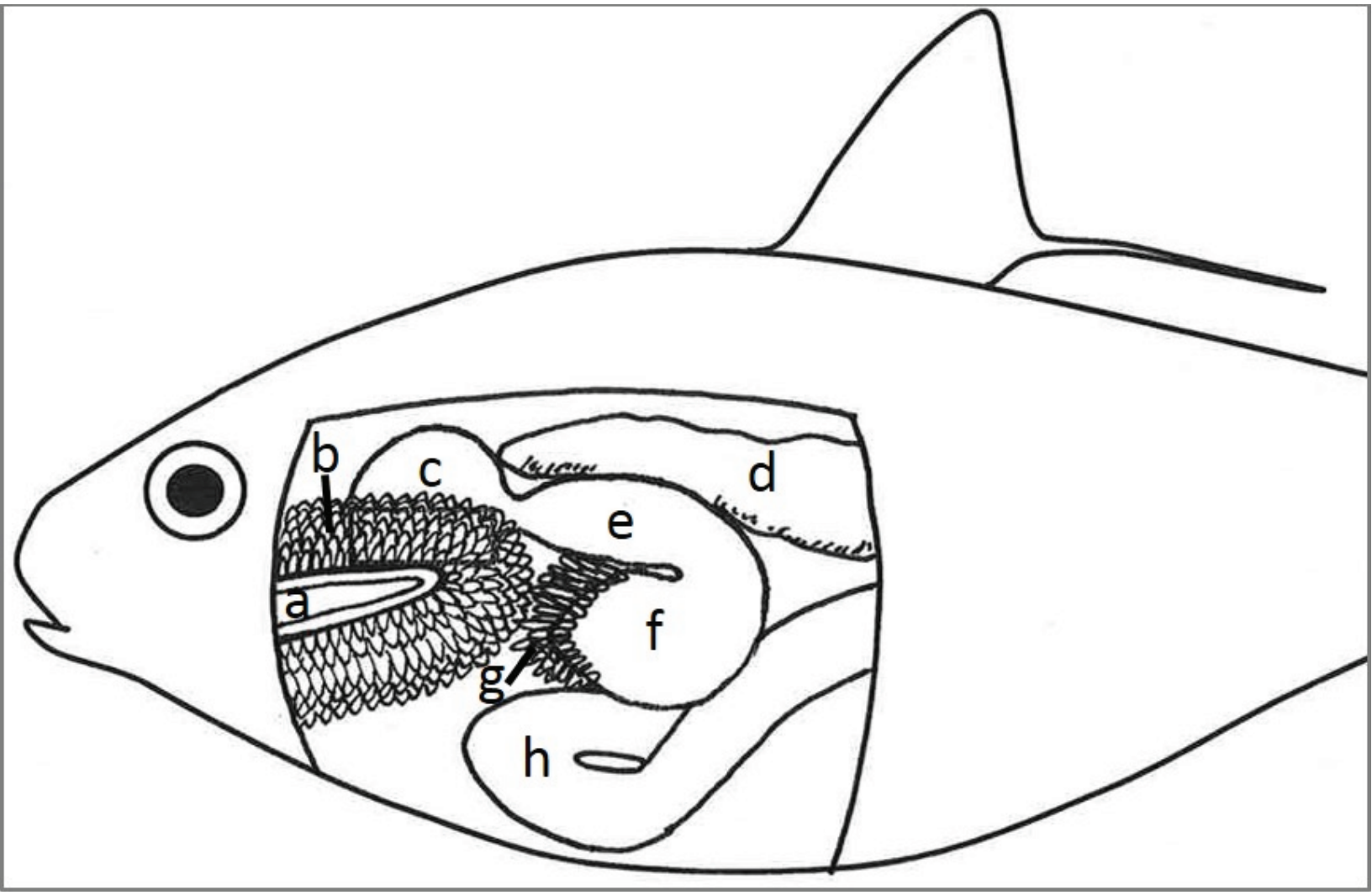
639

Location	% Nitrogen	% Carbon
Water column	3.88 ± 0.31 (11)	29.24 ± 2.32 (11)
Aquarium bottom	2.49 ± 0.28 (11)	18.55 ± 2.47 (11)
Foregut (mucus included)	6.29 ± 0.87 (10)	43.65 ± 10.30 (10)
Foregut (mucus excluded)	5.31 ± 1.07 (10)	43.35 ± 12.70 (10)
Epibranchials (mucus included)	6.25 ± 0.94 (7)	47.45 ± 14.66 (7)
Epibranchials (mucus excluded)	5.53 ± 1.10 (7)	47.38 ± 17.15 (7)
P value, one-way ANOVA, mucus excluded	< 0.0001 $F_{3,35} = 35.02$	< 0.0001 $F_{3,35} = 18.09$

FIGURE CAPTIONS

FIG. 1. *Dorosoma cepedianum* with portion of body wall and operculum removed to illustrate sampling locations and nearby structures. (a) oropharyngeal cavity, (b) gill filaments on gill arches, (c) epibranchial organ, (d) swim bladder, (e) esophagus, (f) gizzard, (e + f) foregut, (g) pyloric caeca and (h) intestine.

FIG. 2. Percent mucus by dry mass in *Dorosoma cepedianum* foregut and epibranchial organs calculated from % nitrogen values obtained in the feeding experiments reported in Table I, using Equations 6 and 7 (mean ± S.E., foregut $n = 9$, epibranchial organs $n = 5$).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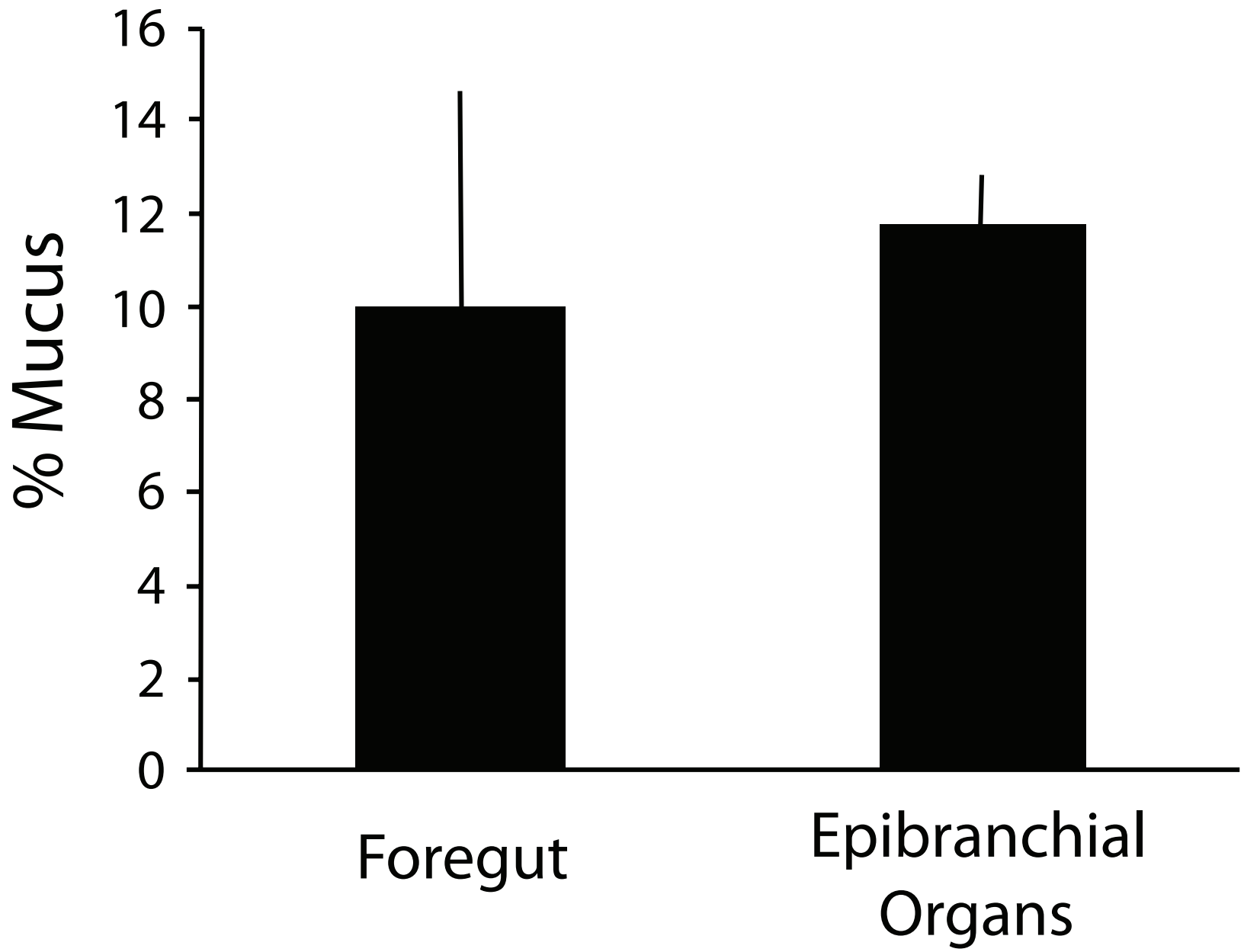
16
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% Mucus
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